

# **Association of HDL Subclasses and Incident Cardiovascular Events: The Jackson Heart and Framingham Offspring Cohort Studies**

*Joshi, Toth, et al. HDL Subclasses and Incident CHD*

## **Supplemental Appendix**

### *Lipoprotein Investigators Collaborative (LIC) Study Group*

The LIC study group is a collaborative effort across four studies including the primary prevention cohorts, the Jackson Heart Study (JHS) and the Framingham Offspring Cohort Study (FOCS), and the secondary prevention cohorts consisting of the Translational Research Investigating Underlying disparities in acute Myocardial infarction Patient's Health status (TRIUMPH) registry and Intermountain Heart Collaborative Study (IHCS) of patients undergoing clinically-indicated coronary angiography. The data for each study are housed at the statistical center for the respective study. The Johns Hopkins Ciccarone Center for the Prevention of Heart Disease serves as the coordinating center for the LIC study group.

### *Vertical Auto Profile (VAP) procedure*

The VAP procedure has been described in detail previously (1-3). Briefly, the procedure consists of three major steps. In the first step, lipoprotein classes and subclasses are separated using a single vertical spin density gradient ultracentrifugation. A two-layer density gradient is prepared in a 5 mL centrifuge tube with the bottom layer consisting of serum diluted 40 fold in 1.21 g/mL potassium bromide solution and the top layer consisting of 1.006 g/mL saline solution. The density gradient is subsequently subjected to a single vertical spin ultracentrifugation at 65,000 rpm for 45 minutes. In the second step, separated lipoprotein fractions are drained from the bottom of centrifuge tube and mixed with cholesterol specific

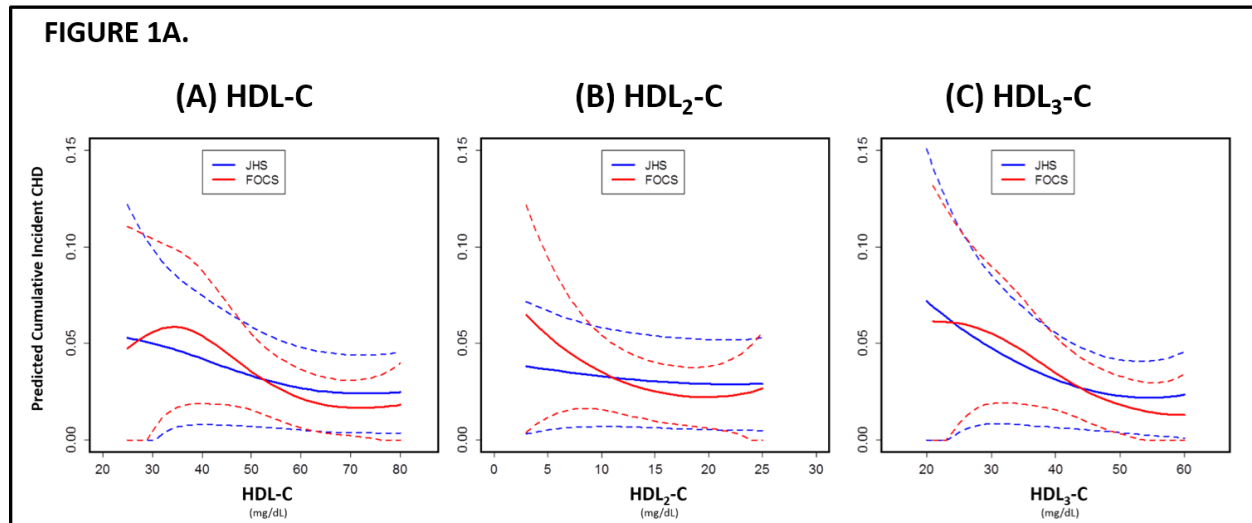
enzymatic reagent using a continuous flow analyzer. The resulting intensity of red color, which is proportional to the concentration of cholesterol in eluting fractions, is measured using a spectrophotometric detector equipped with a flow cell yielding a continuous cholesterol absorbance curve. In the third step, cholesterol concentration of each major lipoprotein class and its subclasses is determined by deconvolution of the main cholesterol absorbance curve using an in-house developed software. Thus, the VAP method provides cholesterol concentration of HDL and its subclasses (HDL<sub>2</sub> and HDL<sub>3</sub>), LDL and its subclasses (LDL<sub>1</sub> through LDL<sub>4</sub>, with LDL<sub>1</sub> being large and the most buoyant and LDL<sub>4</sub> being small and the most dense subclass), Lp(a), IDL, and VLDL and its subclasses (VLDL<sub>1+2</sub>, and VLDL<sub>3</sub>, with VLDL<sub>1+2</sub> being large and the most buoyant and VLDL<sub>3</sub> being small and the most dense subclass). Accuracy of the VAP procedure has been validated by comparing with the standard beta quantification procedure (Core Laboratories for Clinical Studies at Washington University, St. Louis, MO) using split serum specimens. Typically, correlation coefficients for lipoprotein cholesterol between the VAP procedure and beta quantification are: total cholesterol, 0.99; HDL, 0.99; LDL, 0.98; VLDL, 0.98; IDL, 0.80; Lp(a), 0.80; HDL<sub>2</sub>, 0.90; and HDL<sub>3</sub>, 0.90. VAP results are highly reproducible with typical between-days coefficient of variation: total cholesterol, 2.0%; HDL cholesterol, 2.9%; LDL cholesterol, 2.1%; VLDL cholesterol, 2.8%; IDL cholesterol, 8.2%; Lp(a) cholesterol, 9.1%; HDL<sub>2</sub> cholesterol, 9.2%, and HDL<sub>3</sub> cholesterol, 2.5%.

**Supplemental Table 1:** Unadjusted Baseline Cardio-metabolic Risk Factors and Lipids Between Those With and Without Incident CHD in all Jackson Heart Study Participants Including Those Reporting a Prior History of CHD.

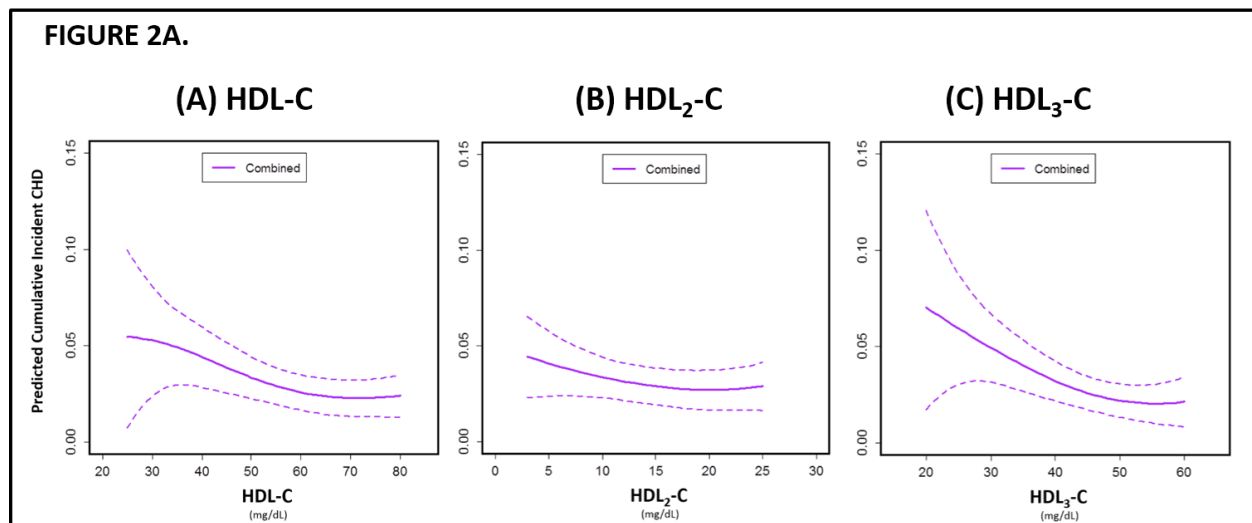
	<b>Jackson Heart Study</b>		
<b>Variable</b>	<b>No CHD (n=4,133)</b>	<b>CHD* (n=125)</b>	<b>p-value</b>
<b>Males</b>	1,471 (36%)	51 (41%)	0.23
<b>Age (years)</b>	53.8 (12.7)	64.8 (9.7)	<0.001
<b>Diabetes</b>	679 (16%)	54 (43%)	<0.001
<b>Current Smoking Status</b>	517 (13%)	18 (14%)	0.53
<b>Body Mass Index (kg/m<sup>2</sup>)</b>	31.8 (7.31)	29.8 (5.9)	0.003
<b>Waist Circumference (cm)</b>	100.5 (16.4)	100.8 (13.3)	0.85
<b>Systolic Blood Pressure (mmHg)</b>	126.5 (18.0)	135.3 (20.1)	<0.001
<b>Diastolic Blood Pressure (mmHg)</b>	79.1 (10.4)	76.9 (11.6)	0.02
<b>Lipid-altering Medications†</b>	454 (11%)	30 (24%)	<0.001
<b>Total Cholesterol (mg/dL)</b>	198.48 (40.3)	202.6 (46.4)	0.26
<b>HDL-C (mg/dL)</b>	53.6 (14.4)	52.1 (14.9)	0.24
<b>HDL<sub>2</sub>-C (mg/dL)</b>	13.5 (6.4)	13.8 (6.4)	0.67
<b>HDL<sub>3</sub>-C (mg/dL)</b>	40.1 (8.7)	38.3 (9.1)	0.03

<b>Direct LDL-C (mg/dL)</b>	122.6 (36.2)	125.9 (39.2)	0.31
<b>Triglycerides (mg/dL)</b>	90 (67,126)	106 (80, 139)	<0.001
<b>Non-HDL-C (mg/dL)</b>	144.9 (38.5)	150.5 (41.2)	0.11
<b>apoAI (mg/dL)</b>	153.0 (23.5)	149.9 (24.9)	0.16
<b>apoB (mg/dL)</b>	97.2 (23.1)	101.8 (24.1)	0.03
<b>TC/HDL-C</b>	3.9 (1.1)	4.1 (1.1)	0.10
<b>apoB/apoAI</b>	0.65 (0.19)	0.69 (0.18)	0.02
Values are n (%), mean (SD), or median (25 <sup>th</sup> , 75 <sup>th</sup> percentile) where appropriate. *p-value based on test for heterogeneity between populations †Lipid-altering Medications include statins, bile sequestrants, niacin derivatives, and fibric acid derivatives			

**Supplemental Figure 1A:** Restricted Cubic Spline Curves Demonstrating the Association of HDL and Subclasses with Risk for Coronary Heart Disease (CHD). Dashed lines represent 95% Confidence Intervals.



**Supplemental Figure 2A:** Restricted Cubic Spline Curves Demonstrating the Association of HDL and Subclasses with Risk for Coronary Heart Disease (CHD) in Meta-Analysis of the Jackson Heart and Framingham Offspring Cohort Studies. Dashed lines represent 95% Confidence Intervals.



## Reference List

- (1) Kulkarni KR, Garber DW, Marcovina SM, Segrest JP. Quantification of cholesterol in all lipoprotein classes by the VAP-II method. J Lipid Res 1994; 35:159-168.
- (2) Kulkarni KR, Marcovina SM, Krauss RM, Garber DW, Glasscock AM, Segrest JP. Quantification of HDL2 and HDL3 cholesterol by the Vertical Auto Profile-II (VAP-II) methodology. J Lipid Res 1997; 38:2353-2364.
- (3) Kulkarni KR. Cholesterol profile measurement by vertical auto profile method. Clin Lab Med 2006; 26:787-802.